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DOI:
10.1097/01.tp.0000280551.78156.ac

Document Version
Early version, also known as pre-print

Citation for published version (Harvard):
Jukes, J-P, Wood, KJ & Jones, ND 2007, 'Natural killer T cells : a bridge to tolerance or a pathway to rejection?', *Transplantation*, vol. 84, no. 6, pp. 679-81. https://doi.org/10.1097/01.tp.0000280551.78156.ac

Natural Killer T Cells: A Bridge To Tolerance Or A Pathway To Rejection?

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Word Counts

Abstract:	**52**
Text:	**1163**
Table/Figure:	**96**

Keywords: NKT Cells, Transplantation, Tolerance, Rejection

Abstract

Over the past twenty years Natural Killer T (NKT) cells have been shown to play an important role in both innate and adaptive immune responses. In this review the potential role of NKT cells in transplantation will be discussed, in particular, their role in rejection and the induction of a state of tolerance.

Natural Killer T (NKT) cells represent a small but significant population of T lymphocytes (<1%) that are reactive to glycolipids presented in the context of the major histocompatibility complex (MHC) class I-like molecule, CD1d (Table 1). Although there appears to be a number of subtypes of NKT cells the most extensively characterised are the invariant or iNKT cells which are defined by the expression of a semi-invariant T cell receptor (TCR) composed of a Vα14-Jα18 chain associated with Vβ8.2, Vβ7, Vβ2 chains in mice and Vα24-Jα18 associated with Vβ11 in humans. Since the discovery of NKT cells in the mid 1980's it has become apparent that these cells have the potential to coordinate sophisticated immune responses, 'bridging' the interface of innate and adaptive immunity (1-3). The focus of this brief review is to discuss the potential role of NKT cells in transplantation immunology, and in particular, their role in rejection and the induction of a state of tolerance.

Upon activation NKT cells can mediate rapid and sustained production of a plethora of cytokines capable of impacting both innate and acquired immune responses (Fig.1A). For example, α-galactosylceramide (αGalCer), to date the most potent iNKT cell ligand, induces cytokine production within hours (4). It is believed that the distribution of NKT cells and the microenvironment in which they exist play a pivotal role in the generation of different effector populations. Indeed in mice NKT cells account for 30% of lymphocytes in the liver and <1% in spleen, blood and lymph nodes.

To date, two models of NKT cell activation have been proposed (5). The first suggests that NKT cells may exist in two subtypes, Th1-like NKT cells and Th2-like NKT cells which preferentially drive Th1 immunity and Th2/tolerogenic responses, respectively. The second model suggests that NKT cells are a homogeneous population that attain functional phenotypes according to the different cytokine microenvironment in which they are activated and the affinity of TCR for the activating ligand (Fig.1A). In addition to the conundrum of generating both pro- and anti-inflammatory cytokines, the downstream leukocyte populations that are activated as a result of NKT cell stimulation adds another level of complexity to the puzzle.

Whilst there is evidence to suggest a role for NKT cells in the immune response to a transplant, it remains unclear whether or not they can recognise alloantigens directly as it has been shown that CD1d,

unlike classical MHC molecules, is not polymorphic (6). NKT cells may be activated during transplantation by the milieu of cytokines generated by non-specific inflammation. Furthermore, the presentation of iGb3, or other as yet unknown glycolipids, following tissue damage may promote activation of NKT cells in a CD1d-dependent manner. NKT cells may promote rejection or tolerance depending on their response to these stimuli,

In two models of streptozotocin induced diabetes, NKT cells have been shown to promote graft loss of intraportally transplanted Islets of Langerhans. Firstly, allogeneic islets transplanted into $NKT^{-/-}$ mice showed a two fold increased survival time compared to those transplanted to WT mice (7). Moreover, in diabetic WT mice normoglycaemia can only be achieved with the transplantation of 400 syngeneic islets, compared to only 100 in $NKT^{-/-}$ mice (8). The process of graft damage was shown to be mediated by interferon-γ (IFNγ) release from NKT cells that subsequently led to the recruitment of neutrophils, resulting in islet loss. However, despite these examples, most available evidence suggests that in the context of transplantation NKT may dampen rather than exacerbate rejection.

Oh *et al*, reported that in $CD1d^{-/-}$ mice transplanted with sex mismatched skin grafts, rejection occurred in an accelerated fashion compared to mice with an intact population of NKT cells. Furthermore, activation of NKT cells with αGalcer significantly increased graft survival in wild-type mice (9). In addition, there are also a number of reports that suggest that NKT cells may facilitate the induction of tolerance to allografts. Seino *et al* (2001) demonstrated that cardiac allografts were accepted in mice treated with anti LFA-1/ICAM-1 or CD28/B7 monoclonal antibodies (mAbs) but were rejected in $iNKT^{-/-}$ mice that received the same mAbs (10). NKT cells have also been reported to be essential for the induction of tolerance in a number of other models, namely, cardiac (11) rat xenograft islets (12) and corneal transplantation (13). However, it should be noted that NKT cell involvement in tolerance induction may be reagent and/or transplant specific as in other models, tolerance induction was unperturbed by the absence of these cells (14).

In all the experimental models outlined above, the beneficial role of NKT cells in prolonging graft survival has been attributed to their production of various cytokines. Skin and cardiac allograft survival was shown to involve increased interleukin-10 (IL-10) production, which was decreased in $CD1d^{-/-}$ mice

(9,11,15). The production of IFNγ by NKT cells remains uncertain, for example, Toyofuku et al found high levels of IFNγ and decreased expression of IL-4 during the rejection process in NKT cell intact mice compared to $NKT^{-/-}$ mice (7). However, IFNγ was found to be required for tolerance induction to cardiac allografts. $NKT^{-/-}$ mice reconstituted with $IFN\gamma^{-/-}$ cells rapidly rejected allografts which survived with the adoptive transfer of NKT cells (10). These data suggest that although there are many gaps in our current understanding of how tolerance may be influenced by NKT cells, there is evidence to suggest that NKT cells may primarily serve to aid the generation of $CD4^{+}CD25^{+}$ T cells with regulatory activity (Treg). Indeed, this has been demonstrated in a model of type-1 diabetes (T1D) where the activation of iNKT with αGalCer conferred protection from T1D which was dependent on IL-4 production and the presence of Treg (17).

Although there is controversy regarding the roles of NKT cells in tolerance and rejection these findings may be explained by subtle changes in the Th1/Th2 bias of NKT cells. Weak antigenicity and therefore weak adaptive responses, may allow NKT greater control of downsteam events, preferentially producing tolerogenic cytokines (9). This may also be the case, as shown in other models where blockade of co-stimulatory, accessory or adhesion molecules is used as a strategy for inducing operational tolerance to allografts. However in cases where a Th1 bias exists and there is a strong allogeneic response, the contribution of NKT cells may be overshadowed as allogeneic T cells dominant the alloantigen driven response (Fig.1B).

The involvement of NKT cells in the immune response after transplantation highlights the function of these dynamic lymphocytes. Whilst it remains unclear as to the precise role of NKT cells in early inflammation, rejection and tolerance, elucidating the precise mechanisms by which they contribute and influence these processes is an important question in modern transplant immunology. There is no doubt that the potential impact NKT cells have on the activation of multiple arms of the immune responses is impressive. It will be important to determine the phenotype and functional properties of each NKT cell population that are responsible for promoting tolerance or rejection and to dissect these signalling pathways in more detail. With the recent discovery of glycolipid analogues that are capable of

preferentially driving NKT cells towards a Th1/Th2 bias, it is an exciting time for understanding how we can drive NKT cells to an altruistic pathway.

Table i.

TCR	Glycosphingolipid Ligands	Cytokine Production	Ref.
NKT Cell TCR **Vα14 Jα18** **associated with** **Vβ8.2, 7, 2**	**α-galactosylceramide (αGalCer)** **∟analogues of αGalCer OCH** **C-glycoside** **Bacterial Recognition** ***Sphingomonas* derived:** **α-glucuronosylceramide (GSL-1)** **α-galacturonosylceramide (GS-1')** **Endogenous Recognition** **Isoglobotrihexosylceramide (iGb3)**	**Th1/Th2 responses (IFNγ, IL-4)** **preferential Th2 response (IL-4)** **preferential Th1 response (IFNγ)** **Th1/Th2 responses (IFNγ, IL-4) but reduced compared to αGalCer**	**(18)** **(19)** **(20)** **(21)** **(22)**

Table i. NKT cells recognise a number of ligands presented by CD1d. These ligands can range from synthetic compounds to bacterial and endogenous glycosphingolipids. Following TCR engagement a variety of Th1 and Th2 cytokines can be released.

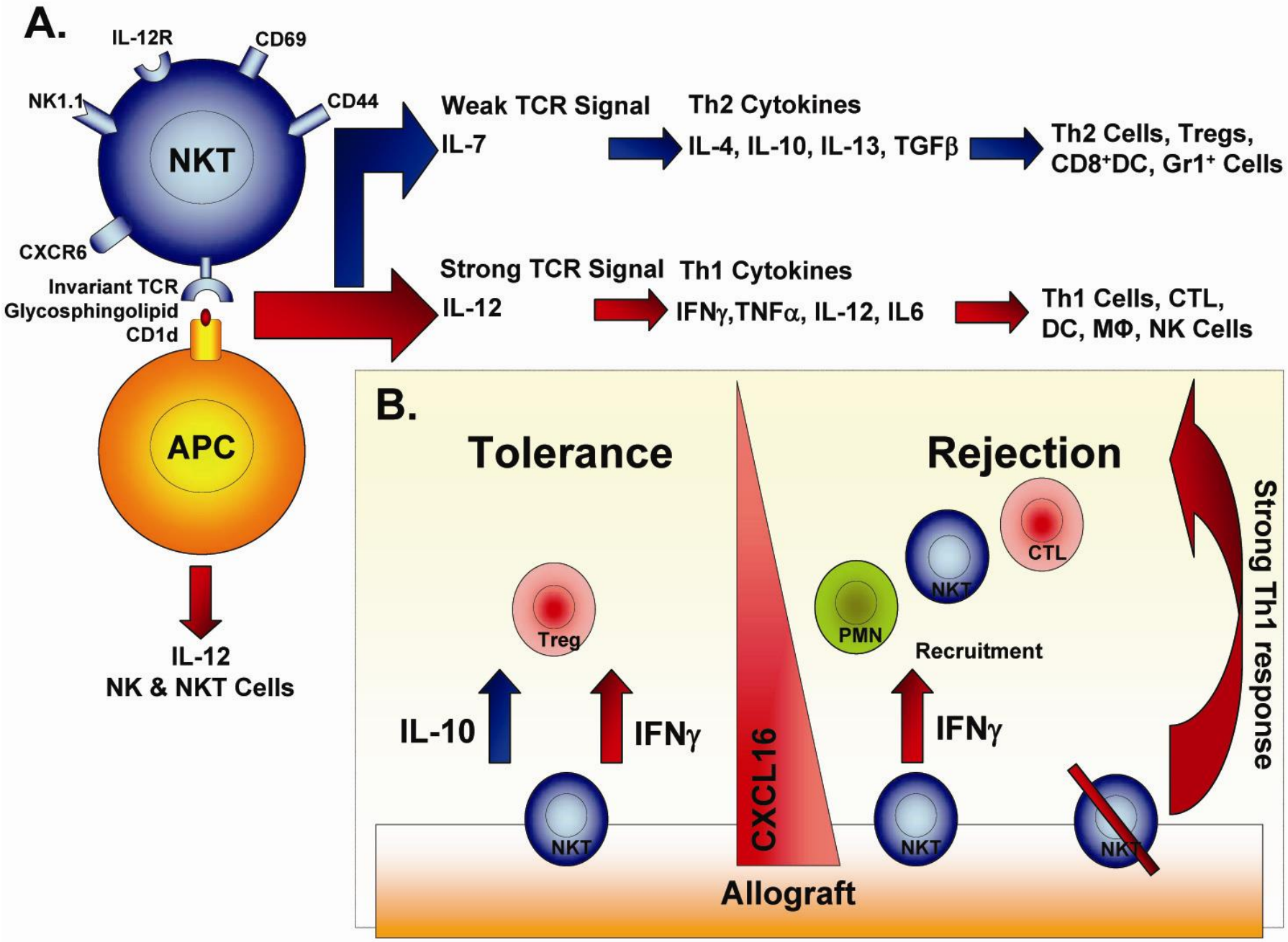
A.
IL-12R
CD69
NK1.1
CD44
NKT
CXCR6
Invariant TCR
Glycosphingolipid
CD1d
APC
IL-12
NK & NKT Cells
Weak TCR Signal
IL-7
Th2 Cytokines
IL-4, IL-10, IL-13, TGFβ
Th2 Cells, Tregs,
CD8+DC, Gr1+ Cells
Strong TCR Signal
IL-12
Th1 Cytokines
IFNγ,TNFα, IL-12, IL6
Th1 Cells, CTL,
DC, MΦ, NK Cells
B.
Tolerance
Rejection
Treg
IL-10
IFNγ
NKT
CXCL16
PMN
NKT
CTL
Recruitment
IFNγ
NKT
NKT
Strong Th1 response
Allograft

Figure 1 A. Activation of NKT cells results in the produce of a number of cytokines which can affect both innate and adaptive immune responses.

Figure 1 B. Following transplantation NKT cells may become activated by increased iGb3 presentation and the local cytokines microenvironment. Other factors that may influence NKT cell activation include the strength of TCR signalling, unknown glycolipid ligands and the site of transplantation.

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